Application of speckle image correlation for real-time assessment of metabolic activity in herpes virus-infected cells

A P Vladimirov[1,2], A S Malygin[1], J A Mikhailova[1,2], E M Borodin[1], A A Bakharev[2] and A P Poryvayeva[2]
[1] Ural Federal University, Yekaterinburg, Mira street ,19, 620002, Russia
[2] FSSI Yekaterinburg Research Institute of Viral Infections of Rospotrebnadzor, Yekaterinburg, Letnaya Street, 23, 620030, Russia

E-mail: vap52@bk.ru

Abstract. Earlier we reported developing a speckle interferometry technique and a device designed to assess the metabolic activity of a cell monolayer cultivated on a glass substrate. This paper aimed at upgrading the technique and studying its potential for real-time assessment of herpes virus development process.
Speckle dynamics was recorded in the image plane of intact and virus-infected cell monolayer. HLE-3, L-41 and Vero cells were chosen as research targets. Herpes simplex virus-1-(HSV-1)-infected cell cultures were studied.
For 24 h we recorded the digital value of optical signal I in one pixel and parameter η characterizing change in the distribution of the optical signal on 10×10-pixel areas. The coefficient of multiple determination calculated by η time dependences for three intact cell cultures equals 0.94. It was demonstrated that the activity parameters are significantly different for intact and virus-infected cells. The difference of η value for intact and HSV-1-infected cells is detectable 10 minutes from the experiment start.

1. Introduction

Human diseases are directly or indirectly associated with cell function impairment. That is why a comprehensive view of normal and pathological cell function mechanisms is vital for both rapid diagnosis of diseases and timely administration of medicines in every individual. Recording laser speckle, or biospeckle, dynamics [1] is a promising tool for studying microscopic processes in biological media. Speckle (a term of an English origin) is a random interference pattern created by mutual interference of multiple coherent waves with random phase shifts. When live objects are illuminated by laser the speckle pattern changes due to temporal changes of scattered wave phases and amplitudes. Speckle dynamics was used to assess the biological activity of seeds [2], fruits [3] and other objects. In [4] speckle contrast imaging was used to assess patients' blood flow in retinae and skin-supplying capillaries of their extremities.

Earlier, a speckle interferometry technique and a device for assessing the metabolic activity of cultivated cells were developed using L-41 cells [5]. The optical difference variation value of the object-probing wave pair path σ_u was selected as a cell activity parametre. High labour input precluding real-time measurements was the disadvantage of the technique.

The objective of this research was upgrading the technique and studying its potential for real-time assessment of herpes virus development process.

2. Experimental procedure

Speckle dynamics was recorded in the image plane of the intact and virus-infected cell monolayer. L-41 CD/84, HLE-3 and Vero cells were selected as the research targets. Herpes simplex virus-1(HSV-1)-infected cell cultures were studied. Experiments were conducted using the device represented in Figure 1.

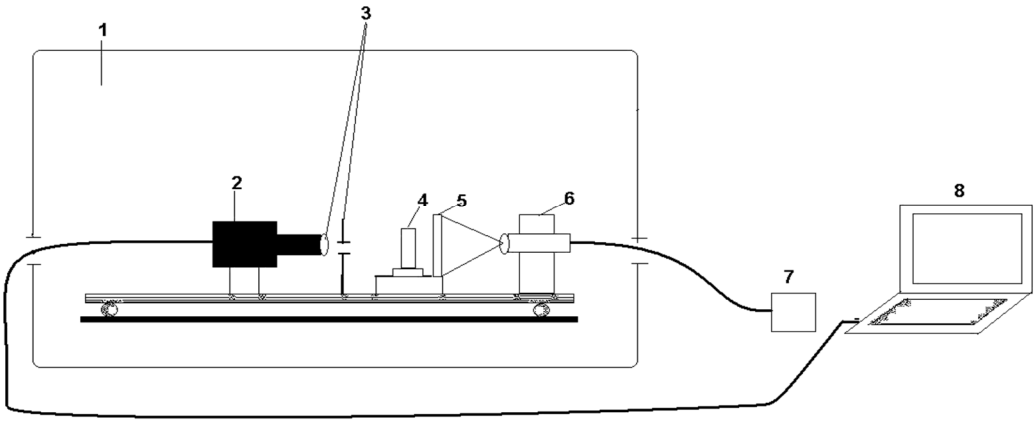

Figure 1. Optical device scheme: 1 – thermostat, 2 – telecamera, 3 – lens with a diaphragm, 4 – tray, 5 – opal glass, 6 – laser module, 7 – line adapter (5V), 8 – PC

For 24 h, we recorded the digital value of optical signal I in one pixel and parameter η characterizing change in the distribution of the optical signal on 10x10-pixel areas. The TV camera exposure time was 9 s, exceeding the radiation intensity correlation time (5-8 s) calculated earlier in [5].

η value was determined using formula (1):

$$\eta = \frac{\frac{1}{m \cdot n}\sum_{i=0}^{m-1}\sum_{j=0}^{n-1}[A_{i,j} - \bar{A}][B_{i,j} - \bar{B}]}{\left(\frac{1}{m \cdot n}\sum_{i=0}^{m-1}\sum_{j=0}^{n-1}[A_{i,j} - \bar{A}]^2\right)^{1/2} \cdot \left(\frac{1}{m \cdot n}\sum_{i=0}^{m-1}\sum_{j=0}^{n-1}[B_{i,j} - \bar{B}]^2\right)^{1/2}}, \quad (1)$$

where $A_{i,j}$ is the digital value of optical signal I in $m \times n$-pixel area at the reference time, $B_{i,j}$ is the digital value of optical signal I in the same area after time τ, I and j are ordinal pixel numbers in the directions of the x and y axes, respectively, $\bar{A}$ is the mean digital value of optical signal I at the reference time, and $\bar{B}$ is the mean digital value of optical signal I after time τ.

Figure 2 represents time dependences of parametre η for HSV-1-infected and intact Vero cells.

Value η tends to decrease gradually for both intact and virus-infected cells. For HLE-3 and L-41 cells, similar dependences were observed. The coefficient of determination (for comparing HSV-1-free and HSV-1-infected cells) was 0.934 for HLE, 0.89 for Vero, and 0.87 for L-41. For three intact cell cultures the coefficient of multiple determination was 0.939. The coefficient value around 1 suggests similarity of the process types in the cells changing the paths of scattered waves. The experimental data analysis showed that the difference of value η for intact and HSV-1-infected cells is detectable 10 minutes from the experiment start.

3. Conclusion

The studies performed may lead to the following conclusions:

1) For various HSV-1-infected and intact cell cultures the time dependences of η have the same trend: value η decreases nonlinearly with increasing experimental time. The coefficient of multiple determination calculated using the dependences $\eta = \eta(\tau)$ for three intact cell types equals 0.94. The coefficient of determination for intact and virus-infected cell cultures is 0.934 for HLE, 0.89 for Vero and 0.87 for L-41 cells.

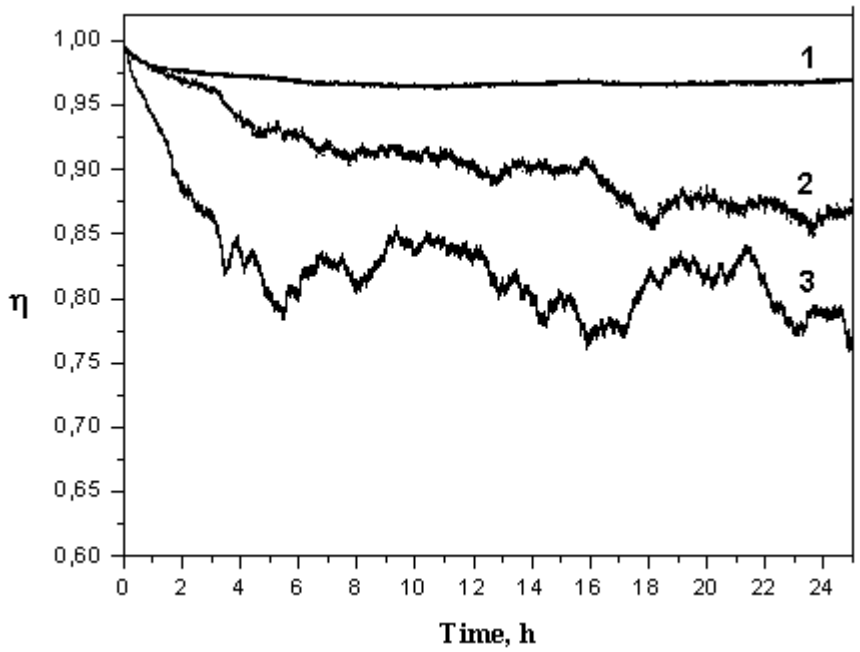

Figure 2. Time dependence of η for Vero cells: 1 – growth medium, 2 – intact cells, 3 – HSV-1-infected cells

2) Time dependences of η for virus-infected and intact cell cultures differ significantly. The difference in value η for intact and HSV-1 infected cells is detectable 10 minutes from the experiment start.

References

[1] *Dynamic Laser Speckle and Applications* 2008 ed Hector J Rabal and Roberto A Braga Jr (New York: CRC Press)

[2] Cardoso R R, Costa A G, Nobre C M B and Braga R A 2011 Frequency signature of water activity by biospeckle laser *Optics Communications* **284** 2131–36

[3] Oulamara, Tribillon G and Duvernoy J 1989 Biological activity measurement on botanical specimen surfaces using a temporal decorrelation effect of laser speckle *Journal of Modern Optics* **36** 165–79

[4] Briers J D 2007 Laser speckle contrast imaging for measuring blood flow *Optica Applicata* **XXXVII** 139-52

[5] Malygin A S, Bebenina N V, Vladimirov A P, Mikitas K N and Baharev A A 2012 A speckle-interferometric device for studying the cell biological activity *Instruments and Experimental Techniques* **55** 415–18